



**UNIVERSITI PUTRA MALAYSIA**

**FACTORS AFFECTING THE OPTIMIZATION OF LIPASE-  
CATALYSED PALM-BASED ESTERS SYNTHESIS**

**ERIN RYANTIN GUNAWAN.**

**FS 2005 35**

**FACTORS AFFECTING THE OPTIMIZATION OF LIPASE-  
CATALYSED PALM-BASED ESTERS SYNTHESIS**

**By**

**ERIN RYANTIN GUNAWAN**

**Thesis Submitted to the School of Graduate Studies,  
Universiti Putra Malaysia, in Fulfillment of the Requirement  
for the Degree of Doctor of Philosophy**

**2005**



## DEDICATION

*I dedicate this thesis to all my family and Ibu Pertiwi.....Indonesia*

Abstract of thesis presented to the Senate of Universiti Putra  
Malaysia in fulfillment of the requirement for the degree of Doctor  
of Philosophy

**FACTORS AFFECTING THE OPTIMIZATION OF LIPASE-  
CATALYSED PALM-BASED ESTERS SYNTHESIS**

By

**ERIN RYANTIN GUNAWAN**

**July 2005**

**Chairman: Professor Mahiran Basri, PhD**

**Faculty: Science**

The lipase synthesis of esters using palm oil fractions and long chain alcohol as substrates was carried out. Various lipases were tested for their suitability for the reaction. Among the enzymes tested, Lipozyme IM exhibited the highest percentage yield of palm oil ester (more than 75%) compared with the other lipases. Two methods, classical method (one-variable at-a-time) and response surface methodology (RSM), were employed for optimization of the reaction.

By the classical method, five parameters such as reaction time, temperature, amount of enzyme, molar ratio of substrates and various organic solvents of the reaction system were investigated.



The optimum yield was achieved at the reaction temperature of 40 - 50°C for palm oil (PO) and 40°C for palm kernel oil (PKO) alcoholysis, a reaction time of 5 – 7 h for PO and 7 – 10 h for PKO, 0.15 g of enzyme for both PO and PKO alcoholysis, molar ratio at 3: 1 (alcohol: PO or PKO), and the best solvent for the reactions was hexane. Percentage yields of esters obtained at these optimum reaction conditions were 83, 80 and 81% for refined, bleached and deodorized (RBD) palm oil, RBD palm stearin (PS) and RBD palm olein (PL), respectively and 87, 90 and 86% for RBD palm kernel oil, RBD palm kernel stearin (PKS) and RBD palm kernel olein (PKL), respectively.

The classical method of optimization involves varying one parameter at a time and keeping the other parameters constant. However, this method is inefficient as it fails to understand relationships between the variables (reaction time, temperature, molar ratio and amount of enzyme) and the response (percentage yield). RSM is an effective statistical technique for the investigation of complex processes. RSM comprising of a five-level, four-factor central composite rotatable design (CCRD) was used to evaluate the interactive effects of synthesis reaction time (2.5 – 10 h of PO, 5 – 15 h of PKO), temperature (30 - 70°C of PO, 30 - 50°C of PKO), amount of enzyme (0.1 - 0.2 g of PO or PKO) and substrates molar ratio (1: 1 - 5: 1 alcohol to PO or PKO) on the percentage yield of

esters and to obtain the optimum conditions for enzyme-catalyzed alcoholysis of palm-based ester.

The optimum conditions derived by RSM of PO and PKO were: reaction time at 7.38 and 10 h, temperature of 53.9°C and 44.2°C, amount of enzyme of 0.149 and 0.157g, and substrates molar ratio 3.41: 1 and 3.78: 1 (alcohol: PO or PKO), respectively. The actual experimental yield of PO and PKO were 84.6% and 90.8% under these optimum conditions, which compared well with the maximum predicted value of 85.4% and 91.8% for PO and PKO, respectively.

The composition of esters synthesized from PO at optimum reaction condition are 0.8% of oleyl laurate, 3.8% of oleyl myristate, 35.5% of oleyl palmitate, 4.5% of oleyl stearate, 33.3% oleyl oleate and 6.2% of oleyl linoleate. Meanwhile, The composition of esters synthesized from PKO are 0.7% of oleyl caproate, 5.7% of oleyl caprylate, 3.7% of oleyl caprate, 36.7% of laurate, 13.10% of oleyl myristate, 8.8% of oleyl palmitate, 2.5% oleyl stearate, 14.1% of oleyl oleate and 2.4% of oleyl linoleate. These optimum conditions were also used in alcoholysis of PS, PL, PKS and PKL, which gave the average percentage yield of more than 80%.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

**FAKTOR-FAKTOR MEMPENGARUHI PENGOPTIMUMAN  
SINTESIS ESTER BERASASKAN KELAPA SAWIT  
DIMANGKINKAN OLEH LIPASE**

Oleh

**ERIN RYANTIN GUNAWAN**

**Julai 2005**

**Pengerusi: Profesor Mahiran Basri, PhD**

**Fakulti: Sains**

Sintesis ester lilin menggunakan pecahan minyak kelapa sawit and alkohol rantai panjang sebagai substrat yang dimangkinkan oleh lipase telah dijalankan. Dua kaedah iaitu kaedah klasik (satu-variase pada satu-masa) dan kaedah permukaan respon, telah digunakan untuk mencari keadaan optima. Pelbagai jenis lipase telah diuji kesesuaiannya untuk tindak balas. Di antara enzim yang telah diuji, Lipozyme IM memberi hasil peratusan ester lilin kelapa sawit paling tinggi (lebih dari 75 %) berbanding lipase lain yang diuji.

Dalam kaedah klasik, lima parameter iaitu masa tindak-balas, suhu, jumlah enzim, nisbah molar substrat dan kepelbagaian pelarut organik telah di kajiselidik.

Hasil optima telah dicapai pada suhu tindak balas alkoholisis 40-50°C bagi minyak kelapa sawit (PO) dan 40°C bagi minyak isirung kelapa sawit (PKO), masa tindak balas pada 5 – 7 jam bagi PO dan pada 7 – 10 jam bagi PKO, jumlah enzim bagi 0.15 g untuk alkoholisis PO dan PKO, nisbah molar substrat pada 3: 1 (alcohol: PO atau PKO) dan pelarut terbaik bagi tindak balas adalah heksana. Hasil peratusan ester lilin yang diperolehi menggunakan keadaan optima ini adalah 83, 80 dan 81% bagi minyak kelapa sawit yang dibersih, diluntur dan dinyahbau (RBD), RBD kelapa sawit sterin (PS) dan RDB kelapa sawit olin, masing-masing 87, 90 dan 86% bagi RBD minyak kelapa sawit (PKL), RBD kelapa sawit isirung sterin (PKS) dan RBD kelapa sawit isirung olin (PKL).

Kaedah klasik bagi pengoptimuman termasuk memvariasikan satu parameter pada satu masa dan mengekalkan selainnya boleh digunakan. Walaubagaimanapun, kaedah ini tidak mencukupi kerana ia gagal untuk memahami hubungkait di antara variable (masa tindak balas, suhu, nisbah molar dan jumlah enzim) dan respon (peratusan hasil). RSM adalah teknik statistik untuk



menyelidik proses yang kompleks. RSM mengandungi lima-peringkat, empat-faktor jenis pusat komposit putaran (CCRD) telah digunakan untuk mengkaji kesan interaktif dalam sintesis bagi masa tindak balas (2.5 – 10h bagi PO, 5 – 15 jam bagi PKO), suhu (30 - 70°C bagi PO, 30 - 50°C bagi PKO), jumlah enzim (0.1 – 0.2 g bagi PO atau PKO) dan nisbah molar susbstrat ( 1: 1 –5: 1 alkohol bagi PO atau PKO) peratusan hasil ester lilin dan untuk mendapatkan keadaan optima untuk alkoholisis bermangkinkan enzim yang menghasilkan ester lilin berasaskan kelapa sawit.

Keadaan optima diperolehi melalui RSM bagi PO dan PKO adalah; masa tindak balas pada 7.38 dan 10 jam, suhu pada 53.9 dan 44.2°C, jumlah enzim pada 0.179 dan 0.157 g dan nisbah molar substrat 3.41: 1 dan 3.78: 1 (alkohol: PO atau PKO), masing-masing. Hasil sebenar dari eksperimen bagi PO dan PKO adalah 84.6% dan 90.8% di bawah keadaan optima, di mana ia sesuai dengan nilai yang dianggar iaitu 85.4% dan 91.8% bagi PO dan PKO. Keadaan optimum ini juga digunakan pada alkoholisis PS, PL, PKS dan PKL, di mana ia telah memberikan purata hasil peratusan lebih daripada 80%.

## ACKNOWLEDGEMENTS

*In The Name of ALLAH, The Most Merciful and Most Beneficent*

All praises do to Allah, Lord of the universe. Only by His grace and mercy this thesis can be completed.

This work was carried out with a hope to contribute towards the expansion of our currently limited knowledge on Oleochemistry. The completion of this thesis would have been impossible if not for the assistance and direct involvement of so many kindhearted individuals. Thus, I am very much indebted to my previous mentors and I have no way of repaying such a debt except to express my sincerest gratitude.

First and foremost, I am very grateful to my adviser Professor Dr. Mahiran Basri, for her strong support, guidance, and patience for the very enriching and thought provoking discussions and lectures which helped to shape the thesis. She was always there to provide everything I needed in the laboratory. I would also like to thank her besides Prof. Dr. Abu Bakar Salleh for providing financial support during the period of study through the IRPA research fund.



I am also grateful to Prof. Dr. Abu Bakar Salleh, Assoc. Prof. Dr. Raja Noor Zaliha Abd. Rahman and Assoc. Prof. Dr. Mohd. Basyaruddin Abd. Rahman in their capacities as members of Supervisory Committee. Thank you for the comments and suggestion, which contributed a lot towards the improvement of the final manuscript. I am also indebted to the staff of the Department of Chemistry, Universiti Putra Malaysia for their help and cooperation.

Special thanks are extended to Dean of Fakultas Keguruan dan Ilmu Pendidikan (FKIP), Rector of Universitas Mataram and Direktorat Pendidikan Tinggi Departemen Pendidikan Nasional Indonesia, which were allowed me to study in PhD level. Special thanks are also extended to other 401 lab members (Dr. Siti Salhah, Salina, Ita, Pei Sin, Azmahani, etc) who helped me in every way possible and providing a congenial and enthusiastic atmosphere in the laboratory. Acknowledgement is also extended to Indonesian Student Association (PPI-UPM) that joined us in sweet friendship and made life easier during my stay in Malaysia.

I wish to express my deepest gratitude to my parents, brothers and sisters for their prayers, continuous moral support and unending encouragement. Last but not least, I wish especially to

acknowledge my beloved husband, Dr. Dedy Suhendra, and my dearest children Erdy Izzatuffikri, Erdanisa Aghnia Ilmani and Erdy Muhammad Fakhri for their love, support, patience and understanding.



I certify that an Examination Committee met on 18<sup>th</sup> July 2005 to conduct the final examination of Erin Ryantin Gunawan on her Doctor of Philosophy thesis entitled "Factors Affecting the Optimization of Lipase-Catalysed Palm-Based Esters Synthesis" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Zulkarnain Zainal, PhD**  
Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Dzulkefly Kuang Abdullah, PhD**  
Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Nor Aripin Shamaan, PhD**  
Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Noorsaadah Abdul Rahman, PhD**  
Professor  
Faculty of Science  
Universiti Malaya  
(External Examiner)



**GULAM RUSUL RAHMAT ALI, PhD**  
Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 AUG 2005

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Mahiran Basri, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Abu Bakar Salleh, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Science  
Universiti Putra Malaysia  
(Member)

**Raja Noor Zaliha Raja Abdul Rahman, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Science  
Universiti Putra Malaysia  
(Member)

**Mohd. Basyaruddin Abdul Rahman, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)



---

**AINI IDERIS, PhD**

Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 08 SEP 2005

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



---

**ERIN RYANTIN GUNAWAN**

DATE: 20/8/2005

## TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xii
DECLARATION	xiv
LIST OF TABLES	xix
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS	xxvi
 <b>CHAPTER</b>	
 <b>I INTRODUCTION</b>	 1
 <b>II LITERATURE REVIEW</b>	 6
Wax Ester	6
Properties of Wax Ester	6
Application of Wax Ester	8
Synthesis of Wax Ester	11
Chemical Synthesis	11
Enzymatic Synthesis	12
Analysis of Wax ester	14
Palm Oil	16
Chemistry of Palm Oil	17
Oleochemicals	21
Palm and Palm Kernel Oils as Raw	21
Material for Oleochemicals	
Lipase as Biocatalyst	22
Lipases in Oleochemical Industry	25
Optimization Studies	26
Response Surface Methodology (RSM)	27
Factorial Design	28
Central Composite Rotatable Design	30
The Four-step Procedure in RSM	31
Application of RSM	34





<b>III</b>	<b>MATERIALS AND METHODS</b>	<b>37</b>
	Materials and Equipments	37
	Methodology	41
	Preparation of Ester Standards	41
	Esterification Reaction	41
	Isolation and Purification of Ester Standards	41
	Synthesis of Esters	42
	Alcoholysis Reaction	42
	Product Isolation and Purification	43
	Product Identification	43
	Thin Layer Chromatography (TLC)	43
	Gas Chromatography (GC)	43
	GC-Mass Spectroscopy Analysis	44
	Fourier Transform Infrared (FTIR) Spectroscopy	45
	Screening of Lipase	45
	Optimization Studies	46
	Effect of Reaction Time	46
	Effect of Reaction Temperature	46
	Effect of Amount of Enzyme	47
	Effect of Molar Ratio of Substrate	47
	Effect of Various Organic Solvents	47
	Kinetic Study	48
	Alcoholysis Reaction at Optimum Condition (Classical Method)	48
	Study on Interactive Effects of Enzymatic Reaction Parameters and Their Optimization Using RSM	49
	Experimental Design	49
	Statistical and Graphical Analyses	54
	Optimization of Reaction and Model Validation	55
	Alcoholysis Reaction at Optimum Condition (RSM)	55
	Enzyme Reusability	56
	The Physical Properties Tests of The Products	56
	Determination of Saponification Value (SV)	56
	Determination of Iodine Value	57
	Determination of Acid Value	59
	Solubility Test	60

<b>IV</b>	<b>RESULTS AND DISCUSSION</b>	<b>61</b>
	Synthesis of Ester	61
	Identification of The Products	62
	Thin Layer Chromatography (TLC)	62
	FTIR Spectroscopy	62
	Gas Chromatography (GC) Analysis	65
	Screening of Enzymes	68
	Optimization Studies of Palm Oil and Palm	71
	Kernel Oil Alcoholysis	
	Effect of Reaction Time	71
	Effect of Reaction Temperature	77
	Effect of Amount of Lipase	82
	Effect of Molar Ratio	86
	Effect of Organic Solvent	91
	Alcoholysis Reaction at Optimum	96
	Conditions	
	Ester Composition of The Products	99
	Kinetic Study	102
	Response Surface Methodology (RSM)	119
	Experimental Design	119
	Fitting Model and ANOVA	124
	Regression Analysis	129
	Parameter Effects	133
	Interactive Effect of Reaction Time and	133
	Temperature	
	Interactive Effect of Substrate Molar	137
	Ratio and Temperature	
	Interactive Effect of Varying Amount of	141
	Enzyme and Temperature	
	Interactive Effect of Reaction Time and	145
	Substrate Molar Ratio	
	Interactive Effect of Reaction Time and	149
	Amount of Enzyme	
	Interactive Effect of Varying Amount of	152
	Enzyme and Substrate Molar Ratio	
	Contour Plots	155
	Optimization of Reaction by RSM	163
	Alcoholysis Reactions at Optimum	166
	Conditions Optimized by RSM	
	Composition of Esters from Palm Oil,	169
	Olein and Stearin Ester Optimized by	
	RSM	
	Comparison of One-variable-at-time	169
	(Conventional) Method and RSM on	
	Percentage Yield of Esters	

Operational Stability	173
Physical Properties of Ester	176
Saponification Value and Molecular Weight	176
Iodine Value (IV)	178
Acid Value (AV)	180
Solubility of The Products	182
Viscosity of The Products	184
<b>V        CONCLUSIONS</b>	<b>186</b>
Recommendation for Further Studies	189
<b>BIBLIOGRAPHY</b>	<b>190</b>
<b>APPENDICES</b>	<b>206</b>
<b>BIODATA OF THE AUTHOR</b>	<b>222</b>

## LIST OF TABLES

Table		Page
1	Physical and chemical properties of palm oil and palm kernel olein	18
2	Fatty acid compositions (%) in palm oils	19
3	Fatty acid compositions (%) in palm kernel oils	19
4	Lipases and their sources	22
5	Coded and Actual Level Combination for a Five-level, Four-Variable Fractional Factorial Design of Palm Oil alcoholysis	48
6	Coded and Actual Level Combination for a Five-level, Four-Variable Fractional Factorial Design of Palm Kernel Oil alcoholysis	50
7	Reactions mixture under optimized condition	53
8	Composition of esters synthesized from palm oil fractions at the optimum condition of palm oil alcoholysis	96
9	Composition of esters synthesized from palm kernel oil fractions at the optimum condition of palm kernel oil alcoholysis	97
10	Kinetic and thermodynamic parameters for the alcoholysis of palm oil/palm kernel oil and oleyl alcohol	110
11	Central composite rotatable quadratic polynomial model, experimental data, actual and predicted values for 5-level-4-factor response surface analysis of palm oil	116

12	Central composite rotatable quadratic polynomial model, experimental data, actual and predicted values for 5-level-4-factor response surface analysis of palm kernel oil	118
13	ANOVA for joint test of palm oil	121
14	ANOVA for joint test of palm kernel oil	124
15	Values and Significance of Regression Coefficients of Coded Factors for Palm Oil Alcoholysis.	127
16	Values and Significance of Regression Coefficients of Coded Factors for Palm Kernel Oil Alcoholysis	128
17	Solutions of optimum conditions of palm oil	160
18	Solutions of optimum conditions of palm kernel oil	161
19	Composition of ester from palm oil fraction alcoholysis at optimum condition optimized by RSM	166
20	Composition of ester from palm kernel oil fraction alcoholysis at optimum condition optimized by RSM	167
21	The saponification value of esters	173
22	The saponification value of esters from different commercial sources	174
23	The iodine values of esters	175
24	The iodine values of commercial esters	176
25	The acid values of esters	177
26	The acid values of commercial esters	177
27	Viscosity of esters	181
28	Viscosity of commercial esters	181

## LIST OF FIGURES

Figure		Page
1	Thin layer chromatography of standard ester catalyzed by Lipozyme	63
2	FTIR spectrum	64
3	Chromatogram of ester standards	65
4	Chromatogram of esters from alcoholysis of palm oil	66
5	Chromatogram of esters from alcoholysis of palm kernel olein	67
6	Screening of lipase	69
7	Effect of reaction time on the total yield of palm oil and palm kernel oil alcoholysis	72
8	Effect of reaction time on palm oil alcoholysis	75
9	Effect of reaction time on palm kernel oil alcoholysis	76
10	Effect of temperature on the total yield of palm oil and palm kernel oil alcoholysis	68
11	Effect of temperature on palm oil alcoholysis	80
12	Effect of temperature on palm kernel oil alcoholysis	81
13	Effect of amount of lipase on the total yield of palm oil and palm kernel oil alcoholysis	83
14	Effect of amount of lipase on palm oil alcoholysis	84
15	Effect of amount of lipase on palm kernel oil alcoholysis	85

16	Effect of molar ratio of substrate on the total yield of palm oil and palm kernel oil alcoholysis	87
17	Effect of molar ratio of substrate on palm oil alcoholysis	89
18	Effect of molar ratio of substrate on palm kernel oil alcoholysis	90
19	Effect of various organic solvents on the total yield of palm oil and palm kernel oil alcoholysis	92
20	Effect of various organic solvents on palm oil alcoholysis	93
21	Effect of various organic solvents on palm kernel oil alcoholysis	94
22	Alcoholysis reaction of palm oil at optimum condition	98
23	Initial reaction rate of alcoholysis as a function of palm oil concentration	104
24	Initial reaction rate of alcoholysis as a function of palm kernel oil concentration	105
25	Initial reaction rate of palm oil alcoholysis as a function of oleyl alcohol concentration	106
26	Initial reaction rate of palm kernel oil alcoholysis as a function of oleyl alcohol concentration	107
27	Lineweaver-Burk plot of alcoholysis as a function of palm oil concentration	110
28	Lineweaver-Burk plot of alcoholysis as a function of palm kernel oil concentration	111
29	Lineweaver-Burk plot of palm oil alcoholysis as a function of oleyl alcohol concentration	112
30	Lineweaver-Burk plot of palm kernel oil alcohol as a function of oleyl alcohol concentration	113

31	Response surface plot showing the effect of incubation time, temperature and their mutual effect on the synthesis of esters from palm oil alcoholysis	135
32	Response surface plot showing the effect of incubation time, temperature and their mutual effect on the synthesis of esters from palm kernel oil alcoholysis	136
33	Response surface plot showing the effect of molar ratio, temperature and their mutual effect on the synthesis of esters from palm oil alcoholysis	139
34	Response surface plot showing the effect of molar ratio, temperature and their mutual effect on the synthesis of esters from palm oil kernel alcoholysis	140
35	Response surface plot showing the effect of amount of enzyme, temperature and their mutual effect on the synthesis of esters of palm oil alcoholysis.	143
36	Response surface plot showing the effect of amount of enzyme, temperature and their mutual effect on the synthesis of esters of palm kernel oil alcoholysis.	144
37	Response surface plot showing the effect of molar ratio, time and their mutual effect on the synthesis of esters from palm oil alcoholysis	147
38	Response surface plot showing the effect of molar ratio, time and their mutual effect on the synthesis of esters from palm oil alcoholysis	148
39	Response surface plot showing the effect of amount of enzyme, time and their mutual effect on the synthesis of esters from palm oil alcoholysis	151



40	Response surface plot showing the effect of amount of enzyme, time and their mutual effect on the synthesis of esters from palm kernel oil alcoholysis.	152
41	Response surface plot showing the effect of amount of enzyme, molar ratio and their mutual effect on the synthesis of esters from palm oil alcoholysis	154
42	Response surface plot showing the effect of amount of enzyme, molar ratio and their mutual effect on the synthesis of esters from palm oil alcoholysis	155
43	Contour plots of percentage yield of esters from alcoholysis of palm oil at molar ratio =1:1-1:5 and amount of enzyme = 0.1g	158
44	Contour plots of percentage yield of esters from alcoholysis of palm oil at molar ratio =1:1-1:5 and amount of enzyme= 0.15 g	159
45	Contour plots of percentage yield of esters from alcoholysis of palm oil at molar ratio =1:1-1:5 and amount of enzyme = 0.2 g	160
46	Contour plots of percentage yield of esters from alcoholysis of palm kernel oil at molar ratio =1:1-1:5 and amount of enzyme = 0.1 g	161
47	Contour plots of percentage yield of esters from alcoholysis of palm kernel oil at molar ratio =1:1-1:5 and amount of enzyme = 0.15 g	162
48	Contour plots of percentage yield of esters from palm kernel oil alcoholysis at molar ratio =1:1-1:5 and amount of enzyme = 0.2 g	163
49	Alcoholysis reaction of palm oils at optimum condition by RSM	168